

Ahmadizar Fariba (Orcid ID: 0000-0002-6261-9445)
Sherwin Catherine (Orcid ID: 0000-0002-0844-3207)

Associations of statin use with glycaemic traits and incident type 2 diabetes

Short running title: Diabetes-risk associated with statin use

Fariba Ahmadizar, PhD¹, Carolina OchoaRosales, MSc^{1,2}, Marja Glisic, PhD^{1,3}, Oscar H Franco, PhD^{1,4}, Taulant Muka, PhD^{1,4}, Bruno H Stricker, PhD^{1,5}

Corresponding author: Prof. dr. Bruno H. Stricker, Department of Epidemiology, Erasmus Medical Centre, PO Box 2040, 3000 CA, Rotterdam, the Netherlands. Email: b.stricker@erasmusmc.nl

The authors confirm that the PI for this paper is Prof. dr. Bruno H. Stricker.

Authors affiliations:

¹Department of Epidemiology, Erasmus University Medical Centre, Rotterdam, the Netherlands.

²Centro de Vida Saludable de la Universidad de Concepción, Concepción, Chile.

³Leibniz Institute for Prevention Research and Epidemiology-BIPS, Bremen, Germany.

⁴Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland.

⁵Department of Internal Medicine, Erasmus University Medical Centre, Rotterdam, the Netherlands.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bcp.13898

Abstract

Aims: There are several epidemiological studies on the association between statins and incident diabetes, but most of them lack details. In this study, we aimed to investigate the association of statin use with glycaemic traits and incident type 2 diabetes.

Methods: Using the prospective population-based Rotterdam Study, we included 9,535 individuals free from diabetes at baseline (>45 years) during the study period between 1997 and 2012. Linear regression analysis was applied to examine the cross-sectional associations between statin use and glycaemic traits including fasting blood serum of glucose and insulin concentrations, and insulin resistance. In a longitudinal follow-up study, we applied a Cox regression analysis to determine adjusted hazard ratios (HR) for incident type 2 diabetes in new users of statins.

Results. The mean age at baseline was 64.3 ± 10.1 year and 41.7% were men. In the fully adjusted model, compared to never users of statins, baseline use of statins was associated with higher concentrations of serum fasting insulin (β , 0.07; 95% CI: 0.02-0.13) and insulin resistance (β , 0.09; 95% CI: 0.03-0.14). Ever use of statins was associated with a 38% higher risk of incident type 2 diabetes (HR, 1.38; 95% CI: 1.09-1.74). This risk was more prominent in subjects with impaired glucose homeostasis and in overweight/obese individuals.

Conclusions: Individuals using statins may be at higher risk for hyperglycaemia, insulin resistance and eventually type 2 diabetes. Rigorous preventive strategies such as glucose control and weight reduction in patients when initiating statin therapy might help minimizing the risk of diabetes.

Key words: Statins, impaired fasting glucose, insulin resistance, type 2 diabetes mellitus.

What is already known about this subject

- Several observational studies and trials have already reported an increased risk of incident type 2 diabetes in subjects treated with statins; however, most of them lack details.
- The increased risk of incident type 2 diabetes associated with statin use is much higher (44%) in observational studies compared with trials (9–13%).
- There are limited studies on the association of statin use with glycemic traits.

What this study adds

Using fully adjusted regression models (adjusted for baseline/post-baseline measurements of potential risk factors):

- Compared to never statin users, baseline use of statin was associated with higher concentrations of serum fasting insulin (β , 0.07; 95% CI: 0.02-0.13) and insulin resistance (β , 0.09; 95% CI: 0.03-0.14).
- Ever use of statins was associated with a 38% higher risk of incident type 2 diabetes (HR, 1.38; 95% CI: 1.09-1.74).
- This risk was more prominent in subjects with impaired glucose homeostasis and in overweight/obese individuals.

Introduction

Although it is well known that statins significantly reduce the risk of cardiovascular disease (CVD) and CVD related mortality [1, 2], statin therapy may lead to increased risk of type 2 diabetes [3, 4]. A recent meta-analysis of 29 randomized clinical trials (RCTs) reported a 12% significantly increased risk of developing type 2 diabetes in the treated arm of statin therapy [5]. However, use under everyday circumstances differs from use in a clinical trial setting with its homogeneous population and short follow-up [6]. Experimental studies suggest that statins may have a diabetogenic effect through beta-cell dysfunction and glucose and insulin secretion/sensitivity [7, 8]; however, the data linking statins with glucose and insulin resistance to show the underlying mechanisms is limited. The literature so far on statin use and the risk of developing type 2 diabetes has been limited by several drawbacks: i) underestimated cases of incident type 2 diabetes due to the inclusion of questionnaire-based data, ii) short follow-up time not adequately taking into account the long-term effects of statins, and iii) lack of a direct comparison between different statin types, dosages and duration of use with respect to diabetes-related outcomes [3, 9, 10].

We hypothesized that statins have influence on serum blood glucose and insulin concentrations and insulin resistance, and lead to hyperglycaemia and hyperinsulinemia as early markers of type 2 diabetes. In this study, exploring different aspects of statin therapy including type, dosages and durations of use, our objective was to assess the population-based association of statins with concentrations of serum fasting glucose and insulin, insulin resistance, and incident type 2 diabetes.

Methods

Study setting. The Rotterdam Study is a prospective population-based cohort study in Ommoord, a district of Rotterdam, the Netherlands. The design of the Rotterdam Study has been described in more detail elsewhere [11]. Briefly, in 1989 all residents aged 45 years or older within the well-defined study area were invited to participate, of whom 78% (7,983 out of 10,275) agreed. In 2000, an additional 3,011 participants were enrolled (RS-II), consisting of all persons living in the study district who had become 55 years of age. A second extension of the cohort was initiated in 2006, in which 3,932 participants aged 45 years or older were included (RSIII). The Rotterdam Study has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants in the present analysis provided written informed consent to participate and to obtain information from their physicians.

Study Design. We performed two analyses. First, a cross-sectional analysis to test the association between statins and glycaemic traits and second, a longitudinal follow-up study to test the association of statins with incident type 2 diabetes.

Population for analysis. The present study used data from the third visit of the first cohort, RSI-3 (March 1997- December 1999) and the baseline examinations of the second cohort, RSII-1 (February 2000-December 2001) and third cohort, RSIII-1 (February 2006-December 2008) (Supplementary **Figure S1**). To study the association between statins and the outcomes of interest, we excluded prevalent type 2 diabetes cases at baseline (n=1,565), and included only participants with available data on statin use and baseline glycaemic values (n=9,535). To study the association of statins with incident type 2 diabetes, patients who used statins at baseline and prevalent cases of cardiovascular diseases (n=691) were also excluded from the

analysis. The end of follow-up for incidence of type 2 diabetes was January 1st 2012 (Supplementary figure 1).

Exposure measurement. All community pharmacies in the Rotterdam Study area store all information on drug dispensing on one common computer network. Information on statin treatment was obtained from the dispensing data using the Anatomical Therapeutic Chemical code (ATC code) (Supplementary table S1). On the baseline date, an individual was considered as current statin user if this date fell within a prescription episode. During follow-up, each participant was classified into one of the following mutually exclusive categories on the event date of type 2 diabetes (in non-cases at the same day of follow-up): 'current use', 'past use', and 'never use'. If the outcome measurement occurred within a prescription episode of statins, a person was classified as a current user and stratified by statin duration into three mutually exclusive groups: <30 days; 31-365 days; and >365 days. If the participant had previously used statins but was no longer a current user on the event date, this was defined as past use. If the participant had not used statin during the study period, this was defined as 'never use' whereas any use ('current' or 'past use') during the study period was defined as 'ever use'.

Outcomes' measurement

Fasting serum glucose concentrations, insulin and insulin resistance. Serum glucose (mmol/l) and insulin (pmol/l) concentrations were measured at the research centre after an overnight fast. For collecting serum specimens from the whole blood, fasting blood samples were centrifuged at 4 °C at 3500 rpm, then serums were immediately frozen at – 140 °C and stored at –80°C in the clinical chemistry laboratory at Erasmus Medical Centre in Rotterdam. Glucose concentration was measured using glucose hexokinase method within 1 week after sampling [12] and insulin concentration by metric assay (Biosource Diagnostics, Camarillo, CA). Homeostasis model assessment-insulin resistance (HOMA-IR) index as a surrogate

marker for the degree of insulin resistance was calculated by the following formula: (fasting serum insulin (mU/l) \times fasting serum glucose (mmol/l))/22.5 [13].

Type 2 diabetes diagnosis. From the date of baseline centre visit and during follow-up, first incident type 2 diabetes cases were identified through active follow-up and according to general practitioners' records, hospital discharge letters, and glucose measurements. Type 2 diabetes was defined as a fasting serum glucose concentration of ≥ 7.0 mmol/l or a non-fasting serum glucose concentration of ≥ 11.1 mmol/l (in case fasting serum samples were not available), or the use of blood glucose-lowering medications [14].

Covariates assessment. At baseline and during follow-up, information was obtained on individuals' characteristics, health status, clinical data including medical and medication history, and behavioural/lifestyle factors. Physical activity levels assessed using the Longitudinal Aging Study Amsterdam Physical Activity Questionnaire expressed in MET-h/week [15] and defined as number of hours per week that participants spent in each activity in the past year. Body mass index (BMI) was calculated as body weight (in kilogram) divided by the square of body height (in metres) included as a continuous variable. Baseline and postbaseline values (at the closest time to diabetes diagnosis) for BMIs were categorized as overweight/obesity defined as $\text{BMI} \geq 25 \text{ kg/m}^2$ versus normal ($\text{BMI} < 25 \text{ kg/m}^2$). Hypertension was defined as an average value (of two measurements) of ≥ 140 mmHg for systolic- and ≥ 90 mmHg for diastolic blood pressure after two measurements and/or a current prescription for an antihypertensive agent. Serum total cholesterol (TC) (mmol/l), high density lipoproteins-cholesterol (HDL-C) (mmol/l), and triglycerides (TG) (mmol/l) were all measured on the COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH). We calculated low density lipoproteins-cholesterol (LDL-C) levels (mmol/l) indirectly from the measurements of TC, HDL-C and TG using the Friedewald equation ($\text{TC} - \text{HDL-C} - \text{TG}/5$) [16]. A family history of diabetes was defined as having a parent, sibling, or both with type 2 diabetes; the

information on family history of diabetes was collected during home visits at RSI and RSII.

All biochemical variables were assessed in serum samples taken after an overnight fasting.

Statistical analyses. For comparison of baseline characteristics between statin users and never users in the first analysis, we used chi-square statistics for dichotomous variables and independent sample t-tests for normally distributed continuous variables. To achieve a symmetric distribution, non-normally distributed data on BMI, TG, fasting serum insulin concentrations and the HOMA-IR index were natural log transformed. Univariable and multivariable linear regression analyses were used to study the association between statins and glycaemic traits including serum fasting glucose and insulin concentrations and HOMA-IR. Correlation coefficients to identify the magnitude of the linear association between serum fasting glucose and insulin concentrations and the HOMA-IR index were studied with the Pearson test.

To study the association between statin use as a time-varying exposure during the follow-up and incident type 2 diabetes, we used multiple Cox regression analyses [17]. The association was estimated by calculating hazard ratio (HR) and 95% confidence intervals (CI). All analyses show the effect estimates for ever, past or current versus never statin users as the reference group. Among ever statin users, in subgroup analyses, we analysed the risk of different outcomes in relation to statin type, dose, and duration of use.

Because statins are often started in patients with type 2 diabetes, we performed two subgroup analyses to avoid confounding by indication. First, we subtracted one year from the event date in type 2 diabetes cases (in non-cases one year from the same date of follow-up) and studied the association with statin use until that date (cumulative statin use). Second, for those participants for whom we had an incident date of impaired fasting glucose (defined as a serum fasting glucose concentration between 6.1 and 6.9 mmol/l), we studied the association between statin use and incident impaired fasting glucose during follow-up.

All regression analyses were adjusted for potential baseline confounding factors using two models including: model 1 adjusted for age and gender, cohort (RSI, II and III), smoking status, alcohol consumption, physical activity, and education level, and model 2 additionally adjusted for BMI and hypertension.

Sensitivity analyses for the association between statin and incident type 2 diabetes. In a sensitivity analysis, we examined the association between statins and risk of incident type 2 diabetes only in a subset of patients (n=6,787) with normal baseline fasting glucose concentrations <6.1 mmol/l by excluding cases of impaired fasting glucose cases (n=2,748). To exclude the likelihood of residual confounding, the association was further adjusted by postbaseline values of BMI. To explore if biological risk factors could partly explain this association, the model 2 was further adjusted for (i) TC, or (ii) HDL-C, LDL-C and TG. In another sensitivity analysis, the model 2 was further adjusted for family history of diabetes, and use of proton pump inhibitors (PPIs) at baseline [18]. To test whether the association between statins and incident type 2 diabetes would be explained by the potential intermediate factors, serum glucose and insulin concentrations were added to the model 2. Using stratified analyses, we also checked the potential effect modification from age at baseline (≤ 65 years versus >65 years), gender and BMI at baseline (overweight/obese (BMI >25) versus normal BMI individuals). We further tested an interaction between statin ever use and serum fasting glucose concentrations using a Cox regression model containing statin ever use, glucose concentrations and the product term of statin ever multiplied by glucose concentrations adjusted for potential confounders in the model 1.

A *p*-value of 0.05 was used to assess the significance of main effect associations. All statistical analyses were carried out using IBM SPSS Statistics software (version 24.0, Chicago, IL, USA).

Results

Baseline characteristics. A total of 9,535 diabetes-free individuals at baseline were included after exclusion of patients with prevalent type 2 diabetes (**Table 1**). The median follow-up for the entire study population was 4 years (a follow-up of up to 15 years.) The mean age at baseline was 64.3 ± 10.1 years and the majority of patients included in this study were females (58.3%). 64.5% of individuals at baseline were overweight/obese. Median serum fasting glucose, insulin concentrations and HOMA-IR index (interquartile range (IQR)) were 5.4 (5.0-5.8), 67.0 (46.0-97.0), and 2.3 (1.5-3.4), respectively.

Characteristics of the baseline statin users and never users are shown in Table 1. Compared to never users, statin users had significantly higher BMIs, TGs, fasting serum glucose and insulin concentrations and insulin resistance and higher prevalence rates of hypertension. Statin users had significantly lower TC, HDL-C and LDL-C as compared to never users. Serum glucose concentrations were weakly but significantly (p -value<0.001) correlated with both (ln) insulin concentrations ($r=0.14$) and (ln) HOMA-IR ($r=0.25$).

After excluding patients who used statins at baseline ($n=968$), the remaining study participants were subsequently followed for a period up to 15 years for incident type 2 diabetes. There were 716 cases of incident type 2 diabetes. In approximately 12.4% of the study population, statins were used during follow-up. In the majority of current statin users, the duration of use was longer than 365 days (75.5%). The most frequently dispensed statins were simvastatin (57.0%), atorvastatin (25.5%), and pravastatin (10.3%).

The association of statin use with glycaemic traits. As shown in Table 2, in a cross-sectional analysis, baseline statin use was statistically significantly associated with increased serum fasting insulin concentrations (β , 0.07; 95%CI: 0.02-0.13) and HOMA-IR index (β , 0.09; 95%CI: 0.03-0.14). However, the association with serum fasting glucose concentrations was no longer significant after adding BMI and hypertension to the model 1 (β , 0.08; 95%CI:

-0.03-0.18). Subgroup multiple linear regression analyses showed no role of statin type and dosage on these associations (**Table 2**).

The association of statin use with incident type 2 diabetes. In a longitudinal follow-up study, compared with never statin users, ever statin use was associated with incident type 2 diabetes (crude HR, 1.64; 95%CI: 1.37-1.97). The observed association remained statistically significant even after adjusting for a range of potential confounders; model 2: HR, 1.38; 95%CI: 1.09-1.74 (**Table 3**). Current but not past use of statins was associated with a greater risk of type 2 diabetes; model 2: HR, 1.52; 95%CI: 1.15-2.00 vs. HR, 1.18; 95%CI: 0.83-1.67.

Among ever statin users, no statistically significant effect of statin type and dosage on risk of incident type 2 diabetes was found. However, we found a significant association of statin duration with incident type 2 diabetes in which longer duration of statin use was associated with a statistically significantly increased risk of incident type 2 diabetes (**Table 3**).

Subgroup analyses

The association of cumulative statin use with incident type 2 diabetes. In a fully adjusted model, cumulative exposure to statins was associated with a 35% higher risk of type 2 diabetes (HR, 1.35; 95% CI: 1.01-1.82). Among statin users, statin dose did not modify the association; HR, 1.12; 95% CI: 0.87-1.44.

The association of cumulative statins with incident impaired fasting glucose. In a Cox regression analysis, cumulative statin use was associated with a 9% higher risk of incident impaired fasting glucose; HR, 1.09; 95% CI: 1.08-1.10 after adjusting for multiple confounders (model 2). Among statin users, in a fully adjusted model, the association between statin dose and incident impaired fasting glucose was statistically significant in

which higher dosages were related to an increased risk of incident impaired fasting glucose; HR, 2.34; 95% CI: 2.32-2.36.

Sensitivity analyses for the association between statin use and incident type 2 diabetes

In a series of sensitivity analyses, when we excluded cases with impaired fasting glucose at baseline, the positive association did not change (crude HR, 1.58; 95% CI: 1.23-2.04); however, when we added BMI and hypertension to the model 1, the association was not statistically significant anymore (HR, 1.28; 95% CI: 1.00-1.76). Using postbaseline values for BMIs did not change the effect estimates. Further adjustment for TC, PPIs, fasting serum glucose and insulin concentrations did not affect the association (**Table 4**). Substituting TC with other blood lipids (HDL-C, LDL-C and TG) attenuated the observed associations. Stratification by age resulted in a statistically significant association only in individuals >65 years (HR, 1.36; 95% CI: 1.02-1.83) but not in those younger than 65 years (HR, 1.25; 95% CI: 0.85-1.84). In gender-stratified analyses, the association between statin use and risk of incident type 2 diabetes was only statistically significant in males (HR, 1.52; 95% CI: 1.07-2.16) but not in females (HR, 1.28; 95% CI: 0.93-1.74). When we stratified the analysis by baseline BMI, our findings showed a statistically significant association only in a subset of overweight/obese subjects (HR, 1.42; 95% CI: 1.10-1.83) but not in those with normal BMI (HR, 1.18; 95% CI: 0.69-2.02). The interaction term of statin ever use with serum fasting glucose concentrations was highly significant exerting the highest odds of incident type 2 diabetes (HR, 3.51; 95% CI: 2.51-4.91).

Discussion

To the best of our knowledge, this is the first detailed population-based study to show the effect of statin use on incident type 2 diabetes in those free from diabetes at baseline. At baseline, statin use was associated with elevated values for several glycaemic traits. After

excluding these baseline statin users, new ever statin users during a follow-up of up to 15 years showed a 38% increased risk of incident type 2 diabetes independent of statin type and dosage. All observed associations between statin use and incident type 2 diabetes remained significant even after adjusting for several potential risk factors for diabetes, e.g. age, physical activity and education level. However, there was a steep decrease in the effect estimates corresponding to the adjusted models.

Impaired glycaemic traits are important hallmarks of incident diabetes in which impaired glucose metabolism and decreased insulin sensitivity are the two major pathophysiological disturbances required for the conversion to overt type 2 diabetes [19]. Currently, limited evidence is available concerning the impact of statin use on glycaemic traits [20, 21]. Elevated baseline serum glucose and insulin concentrations might be explained by a diabetogenic effect of statins. However, conclusions from previous studies on this effect is inconsistent [22-24]. Theoretically, some potential underlying mechanisms for this effect in diabetes-free individuals consist of i) decreased insulin-mediated cellular glucose uptake leading to glucose intolerance, ii) decreased isoprenoids synthesis which causes downregulation of glucose transporter 4 and leads to hyperglycaemia and hyperinsulinemia, and iii) reduced factors such as coenzyme Q10, farnesyl pyrophosphate, and dolichol leading to altered insulin secretion/resistance [25-28]. Since the association of statin use and serum fasting glucose concentrations was no longer significant after including BMI and hypertension, our finding suggests that the association between statins and diabetes could be through insulin secretion/resistance.

In our study, the significantly higher risk of incident type 2 diabetes among statin users confirms and extends the results of previous studies [6, 24, 29]. Several observational studies and RCTs have already reported an increased risk of incident type 2 diabetes in subjects treated with statins; where the increased risk reported by observational studies is

much higher (44%) compared with RCTs (9–13%) [6, 24]. Discrepant results might be explained by the much longer follow-up time in observational studies than in RCTs and a relatively less healthy population as trials preferably enrol participants without multimorbidity. Longer time could result in an increased probability of developing adverse drug events such as diabetes [29]. According to the findings of a previous study including over 2 million subjects in the UK, the statin-associated diabetes risk (HR=1.22) over a period of 3 years was increased with longer statin duration in which the strongest significant association was observed when patients were followed-up for 15–20 years (HR=3.63) [30]. The magnitude of the association between statins and incident type 2 diabetes is also partly dependent on different confounders such as lipid profile and family history of diabetes [31]. In the 3 large RCTs, baseline fasting glucose concentrations and features of the metabolic syndrome e.g. TG were shown as predictive factors of incident type 2 diabetes among statin users [32]. Similarly, we showed that the association between statin therapy and incident type 2 diabetes was partly dependent on lipid profiles including HDL-C, LDL-C and TG, though, independent of several diabetes risk factors such as hypertension. Moreover, our result of the interaction between statin use and serum fasting glucose concentrations was highly significant implying the greatest statin-associated diabetes risk in presence of baseline impaired glucose concentrations. Our finding here is consistent with the results of JUPITER trial where the majority of statin users who developed diabetes during 5 years of follow-up had already impaired fasting glucose. However, this study was restricted to rosuvastatin at a single dosage of 20 mg daily [33].

In our study, the majority of patients were on lipid-soluble statins (simvastatin, atorvastatin and fluvastatin) while the number of patients on water-soluble statins (pravastatin) was low (10.3%). Therefore, due to different actions of specific molecules of statins on insulin resistance and metabolic risk profiles, further studies are required. Our

findings concerning a significantly increased risk of incident type 2 diabetes independent of statin types and dosages are in line with a previous meta-analysis [34]. To date, limited evidence suggests that different statin therapy including different types, dose and duration might exert distinct effects on statin related outcomes and the results reported by previous studies are conflicting [6, 34]. In line with our hypothesis of a cumulative effect of statins on impaired glucose and insulin resistance, the risk of incident diabetes was significantly higher in intermediate (31-365 days)- and long-term (>365 days) users of statins.

We showed a significantly increased risk of statin-related type 2 diabetes only among overweight/obese but not in individuals with a normal BMI. Given the concern that BMI values above normal and dyslipidaemia are both associated with the onset of type 2 diabetes [35], it is important to focus on more effective prevention in individuals with a high risk taking this medication. This could also help improving their lipid profile and thereby reducing the risk of cardiovascular diseases.

Our study has some strengths and limitations. First, the population-based and prospectively gathered information on disease outcome make selection- and information bias unlikely. The long follow-up, the availability of data on statin type, dose and duration of use along with the use of a series of adjusted regression models with a broad range of potential confounders are among the most important strengths in this study. Moreover, we were able to provide a comprehensive overview of the associations between statins and glycaemic traits and incident type 2 diabetes. However, our study has some potential limitations that should be acknowledged. Most importantly, to study the association between statins and incident type 2 diabetes, we excluded prevalent type 2 diabetes cases at baseline (n=1,565), and included only participants with normal fasting glucose levels (<6.1 mmol/l) who were not on hypoglycaemic medication. Unfortunately, we did not have data on HbA1c at baseline, a marker with the ability of reflecting the long-term glycemic history. Also, the association

between statins and type 2 diabetes could be caused by reverse causation in which high risk individuals e.g. patients with obesity and fatty liver were prescribed statins to prevent CVD. However, to decrease the effect of reverse causality on our findings, we performed two subgroup analyses including the associations of type 2 diabetes with cumulative statin use until one year before diabetes and an impaired fasting glucose, where the results still showed significant associations. Cumulative statin users during follow-up showed a 9% increased risk of incident impaired fasting glucose in which the magnitude of increased risk was directly proportional to the statin dosage. Though, we were able to adjust the associations for many measured confounding variables, the possibility remains that some factors e.g. genetic factors which we have not measured still modify the associations [36]. Additionally, compared with never statin users, patients treated with statins tend to be sicker and are more prone to enhanced glucose and insulin concentrations, and to eventually developing diabetes [37]. We cannot rule out the effect of detection bias in our study in which individuals treated with statins are more likely to be clinically evaluated. Finally, since our study included a population consisting of roughly 95% Caucasian ethnicity and race might be an important factor for the association between statin use and susceptibility of diabetes, our findings cannot be easily generalized to other populations.

In summary, using multiple subgroup and sensitivity analyses, we found a consistent significant association between statin use and incident type 2 diabetes. Our analysis also highlights the greatest effect of statins in presence of hyperglycaemia and overweight/obesity. This suggests that it is necessary to take statin diabetogenicity into consideration in clinical practice when statin is indicated, emphasising the concomitant need for dietary measures and exercise.

Acknowledgments. The contribution of the study participants, general practitioners and pharmacists of the Ommoord district to the Rotterdam Study is gratefully acknowledged.

References

1. Baigent C, Landray M, Reith C, et al. Study of heart and renal protection (SHARP): Randomized trial to assess the effects of lowering low-density lipoprotein cholesterol among 9,438 patients with chronic kidney disease. *Am Heart J*. 2010;160(5):785-U28.
2. Cholesterol Treatment Trialists' (CTT) Collaborators, Kearney PM, Blackwell L, Collins R, Keech A, Simes J, Peto R, Armitage J, Baigent C. Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: A meta-analysis. *Lancet* 2008 Jan 12;371(9607):117-125
3. Culver AL, Ockene IS, Balasubramanian R, et al. Statin use and risk of diabetes mellitus in postmenopausal women in the women's health initiative. *Arch Intern Med* 2012 Jan 23;172(2):144-152
4. Cederberg H, Stancakova A, Yaluri N, Modi S, Kuusisto J, Laakso M. Increased risk of diabetes with statin treatment is associated with impaired insulin sensitivity and insulin secretion: A 6 year follow-up study of the METSIM cohort. *Diabetologia* 2015 May;58(5):1109-1117
5. Thakker D, Nair S, Pagada A, Jamdade V, Malik A. Statin use and the risk of developing diabetes: A network meta-analysis. *Pharmacoepidemiol Drug Saf* 2016 Oct;25(10):1131-1149
6. Casula M, Mozzanica F, Scotti L, et al. Statin use and risk of new-onset diabetes: A meta-analysis of observational studies. *Nutr Metab Cardiovasc Dis* 2017 May;27(5):396-406
7. Chen YH, Chen YC, Liu CS, Hsieh MC. The different effects of atorvastatin and pravastatin on cell death and PARP activity in pancreatic NIT-1 cells. *J Diabetes Res* 2016;2016:1828071
8. Lorza-Gil E, Salerno AG, Wanschel AC, et al. Chronic use of pravastatin reduces insulin exocytosis and increases beta-cell death in hypercholesterolemic mice. *Toxicology* 2016 Feb 17;344-346:42-52
9. Rajpathak SN, Kumbhani DJ, Crandall J, Barzilai N, Alderman M, Ridker PM. Statin therapy and risk of developing type 2 diabetes: A meta-analysis. *Diabetes Care* 2009 Oct;32(10):1924-1929
10. Sattar N, Preiss D, Murray HM, et al. Statins and risk of incident diabetes: A collaborative meta-analysis of randomised statin trials. *Lancet* 2010 Feb 27;375(9716):735-742
11. Ikram MA, Brusselle GGO, Murad SD, et al. The rotterdam study: 2018 update on objectives, design and main results. *Eur J Epidemiol* 2017 Oct 24
12. Neeley WE. Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6-phosphate dehydrogenase method. *Clin Chem* 1972 Jun;18(6):509-515

13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 Jul;28(7):412-419
14. Ligthart S, van Herpt TT, Leening MJ, et al. Lifetime risk of developing impaired glucose metabolism and eventual progression from prediabetes to type 2 diabetes: A prospective cohort study. *Lancet Diabetes Endocrinol* 2016 Jan;4(1):44-51
15. Stel VS, Smit JH, Pluijm SM, Visser M, Deeg DJ, Lips P. Comparison of the LASA physical activity questionnaire with a 7-day diary and pedometer. *J Clin Epidemiol* 2004 Mar;57(3):252-258
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972 Jun;18(6):499-502
17. Stricker BH, Stijnen T. Analysis of individual drug use as a time-varying determinant of exposure in prospective population-based cohort studies. *Eur J Epidemiol* 2010 Apr;25(4):245-251
18. Lin HC, Hsiao YT, Lin HL, et al. The use of proton pump inhibitors decreases the risk of diabetes mellitus in patients with upper gastrointestinal disease: A population-based retrospective cohort study. *Medicine (Baltimore)* 2016 Jul;95(28):e4195
19. Singh B, Saxena A. Surrogate markers of insulin resistance: A review. *World J Diabetes* 2010 May 15;1(2):36-47
20. Sukhija R, Prayaga S, Marashdeh M, et al. Effect of statins on fasting plasma glucose in diabetic and nondiabetic patients. *J Investig Med* 2009 Mar;57(3):495-499
21. Parida S, Swain TR, Routray SN, Maiti R. Effect of atorvastatin on glycaemic parameters in normoglycaemic and prediabetic subjects: A prospective, panel study. *J Clin Diagn Res* 2017 Feb;11(2):FC04-FC09
22. Kostapanos MS, Milionis HJ, Agouridis AD, Rizos CV, Elisaf MS. Rosuvastatin treatment is associated with an increase in insulin resistance in hyperlipidaemic patients with impaired fasting glucose. *Int J Clin Pract* 2009 Sep;63(9):1308-1313
23. Chan DC, Pang J, Watts GF. Pathogenesis and management of the diabetogenic effect of statins: A role for adiponectin and coenzyme Q10?. *Curr Atheroscler Rep* 2015 Jan;17(1):472-014-0472-7
24. Millan Nunez-Cortes J, Cases Amenos A, Ascaso Gimilio JF, et al. Consensus on the statin of choice in patients with impaired glucose metabolism: Results of the DIANA study. *Am J Cardiovasc Drugs* 2017 Apr;17(2):135-142
25. Wang S, Cai R, Yuan Y, Varghese Z, Moorhead J, Ruan XZ. Association between reductions in low-density lipoprotein cholesterol with statin therapy and the risk of new-onset diabetes: A meta-analysis. *Sci Rep* 2017 Jan 10;7:39982
26. Kanda M, Satoh K, Ichihara K. Effects of atorvastatin and pravastatin on glucose tolerance in diabetic rats mildly induced by streptozotocin. *Biol Pharm Bull* 2003 Dec;26(12):1681-1684

27. Andersson C, Lyass A, Larson MG, Robins SJ, Vasan RS. Low-density-lipoprotein cholesterol concentrations and risk of incident diabetes: Epidemiological and genetic insights from the framingham heart study. *Diabetologia* 2015 Dec;58(12):2774-2780
28. Chen WM, Sheu WH, Tseng PC, et al. Modulation of microRNA expression in subjects with metabolic syndrome and decrease of cholesterol efflux from macrophages via microRNA-33-mediated attenuation of ATP-binding cassette transporter A1 expression by statins. *PLoS One* 2016 May 3;11(5):e0154672
29. Mansi I, Frei CR, Wang CP, Mortensen EM. Statins and new-onset diabetes mellitus and diabetic complications: A retrospective cohort study of US healthy adults. *J Gen Intern Med* 2015 Nov;30(11):1599-1610
30. Macedo AF, Douglas I, Smeeth L, Forbes H, Ebrahim S. Statins and the risk of type 2 diabetes mellitus: Cohort study using the UK clinical practice research datalink. *BMC Cardiovasc Disord* 2014 Jul 15;14:85-2261-14-85
31. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB S. Prediction of incident diabetes mellitus in middle-aged adults: The framingham offspring study. *Arch Intern Med* 2007 May 28;167(10):1068-1074
32. Waters DD, Ho JE, DeMicco DA, et al. Predictors of new-onset diabetes in patients treated with atorvastatin: Results from 3 large randomized clinical trials. *J Am Coll Cardiol* 2011 Apr 5;57(14):1535-1545
33. Ridker PM, Pradhan A, MacFadyen JG, Libby P, Glynn RJ. Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: An analysis from the JUPITER trial. *Lancet* 2012 Aug 11;380(9841):565-571
34. Navarese EP, Buffon A, Andreotti F, et al. Meta-analysis of impact of different types and doses of statins on new-onset diabetes mellitus. *Am J Cardiol* 2013 Apr 15;111(8):1123-1130
35. Abhay kumar pandey , deepti pandey , abha pandit. obesity and lipid profile study in type 2 diabetes patients with auditory and reaction time deficits and non-diabetic control subjects. *advances in diabetes and metabolism*. 2017; 5 , 1 - 5. doi: 10.13189/adm.2017.050101.
36. Stancakova A, Kuulasmaa T, Kuusisto J, et al. Genetic risk scores in the prediction of plasma glucose, impaired insulin secretion, insulin resistance and incident type 2 diabetes in the METSIM study. *Diabetologia* 2017 Sep;60(9):1722-1730
37. Schrom JR, Caraballo PJ, Castro MR, Simon GJ. Quantifying the effect of statin use in pre-diabetic phenotypes discovered through association rule mining. *AMIA Annu Symp Proc* 2013 Nov 16;2013:1249-1257

Table 1. Baseline characteristics of the study population

	Total (n=9,535)	Statin users (n=968)	Never statin users (n=8,567)	P-value
Age, years, mean \pm SD	64.3 \pm 10.1	64.9 \pm 8.2	64.3 \pm 10.2	0.04
Gender (male), n (%)	3977 (41.7)	481 (49.7)	3496 (40.8)	<0.001
BMI, kg/m ² , mean \pm SD	27.0 \pm 4.1	27.5 \pm 3.9	26.9 \pm 4.1	<0.001
Family history of diabetes, n (%)	524 (8.7)	53 (5.5)	471 (5.5)	0.98
Hypertension, n (%)	3472 (36.5)	651 (67.3)	2821 (32.9)	<0.001
Total cholesterol, mmol/l, median (IQR)	5.7 (5.1-6.4)	5.1 (4.4-5.7)	5.8 (5.2-6.5)	<0.001
HDL-C, mmol/l, median (IQR)	1.4 (1.1-1.7)	1.3 (1.1-1.5)	1.4 (1.2-1.7)	<0.001
LDL-C, mmol/l, median (IQR)	4.0 (3.4-4.6)	3.4 (2.8-3.9)	4.1 (3.5-4.7)	<0.001
Triglycerides, mmol/l, median (IQR)	1.3 (1.0-1.8)	1.5 (1.1-2.1)	1.3 (0.97-1.7)	<0.001
Physical activity, MET-h/week, mean \pm SD	75.6 \pm 49.6	75.4 \pm 47.2	75.7 \pm 49.8	0.44
Education status (High), n (%)	1717 (18.0)	153 (15.8)	1564 (18.3)	0.05
Smoking status (ever), n (%)	2040 (21.4)	222 (22.9)	1818 (21.2)	0.28
Alcohol consumption (ever), n (%)	5443 (57.1)	539 (55.7)	4904 (57.2)	0.32
Glucose, mmol/l, median, IQR	5.4 (5.0-5.8)	5.5 (5.1-5.9)	5.4 (5.0-5.8)	0.02
Insulin, pmol/l, median, IQR	67.0 (46.0-97.0)	78.0 (54.0-115.0)	66.0 (46.0-96.0)	<0.001
HOMA-IR index, median, IQR	2.3 (1.5-3.4)	2.7 (1.8-4.1)	2.3 (1.5-3.4)	<0.001
Type of statins				
Simvastatin (C10AA01)	529 (57.0)	-	-	-
Atorvastatin (C10AA05)	237 (25.5)	-	-	-
Pravastatin (C10AA03)	96 (10.3)	-	-	-
Fluvastatin (C10AA04)	66 (7.1)	-	-	-
Average statin dose, mg				
Simvastatin (C10AA01)	36	-	-	-
Atorvastatin (C10AA05)	42	-	-	-
Pravastatin (C10AA03)	33	-	-	-
Fluvastatin (C10AA04)	54	-	-	-

Abbreviations: SD, standard deviation; IQR, interquartile range; HDL-C, high density lipoproteins-cholesterol; LDL-C, low density lipoproteins-cholesterol; HOMA-IR, homeostasis model assessment-Insulin resistance

Table 2. Multivariate linear regression analysis on the association between statin therapy and serum fasting glucose/insulin concentrations and HOMA-IR

	Crude effect	Model 1	Model 2
Glucose concentration	β coefficient (SEM); 95% CI	β coefficient (SEM); 95% CI	β coefficient (SEM); 95% CI
Baseline statin users	0.12 (0.07); -0.02-0.25	0.14 (0.06); 0.04-0.27	0.08 (0.06); -0.03-0.18
Subgroup analyses			
Statin type			
Simvastatin	0.03 (0.09); -0.14-0.20	-0.01 (0.06); -0.14-0.11	0.01 (0.05); -0.08-0.11
Atorvastatin	-0.10 (0.10); -0.29-0.10	0.02 (0.08); -0.13-0.18	-0.06 (0.06); -0.17-0.06
Others*	Ref		
Statin dose	-0.13 (0.07); -0.27-0.01	0.02 (0.06); -0.09-0.14	-0.07 (0.04); -0.16-0.01
(ln) Insulin concentration			
Baseline statin users	0.15 (0.02); 0.11-0.20	0.13 (0.03); 0.08-0.19	0.07 (0.03); 0.02-0.13
Subgroup analyses			
Statin type			
Simvastatin	0.01 (0.04); -0.07-0.09	0.08 (0.05); -0.02-0.18	0.05 (0.05); -0.04-0.15
Atorvastatin	0.11 (0.05); 0.01-0.20	0.05 (0.06); -0.08-0.17	0.05 (0.06); -0.07-0.16
Others*	Ref		
Statin dose	0.19 (0.03); 0.13-0.25	0.03 (0.05); -0.06-0.12	-0.01 (0.04); -0.09-0.08
(ln) HOMA-IR			
Baseline statin users	0.19 (0.02); 0.14-0.23	0.16 (0.03); 0.10-0.21	0.09 (0.03); 0.03-0.14
Subgroup analyses			
Statin type			
Simvastatin	0.01 (0.04); -0.07-0.10	0.08 (0.05); -0.03-0.19	0.05 (0.05); -0.04-0.15
Atorvastatin	0.10 (0.05); -0.02-0.19	0.04 (0.07); -0.09-0.17	0.04 (0.06); -0.08-0.16
Others*	Ref		
Statin dose	0.19 (0.04); 0.12-0.25	0.03 (0.05); -0.07-0.13	-0.02 (0.05); -0.11-0.07

Abbreviation: SEM, standard error of the mean; Ln, natural logarithm; CI, confidence interval; HOMA-IR, homeostasis model assessment-insulin resistance.

Model 1 adjusted for age, gender, cohort (I, II and III), smoking status, alcohol consumption, physical activity, and education level, and model 2 included all variables in model 1 addition to body mass index and hypertension.

*Others including pravastatin and fluvastatin.

Table 3. Univariate and multivariate Cox regression analysis on the associations of statin use with incident type 2 diabetes

	Crude effect: HR; 95% CI	Model 1: HR; 95% CI	Model 2: HR; 95% CI
Type 2 diabetes (n=8,844)			
<i>Ever statin users</i>	1.64; 1.37-1.97	1.49; 1.19-1.86	1.38; 1.09-1.74
<i>Past statin users</i>	1.44; 1.10-1.89	1.20; 0.85-1.68	1.18; 0.83-1.67
<i>Current statin users</i>	1.63; 1.32-2.02	1.61; 1.23-2.10	1.52; 1.15-2.00
Subgroup analyses-among ever statin users			
<i>Statin type</i>			
Simvastatin	0.98; 0.65-1.50	0.92; 0.55-1.53	0.91; 0.54-1.55
Atorvastatin	1.09; 0.69-1.72	1.50; 0.88-2.56	1.73; 0.99-3.01
Others*	Ref		
<i>Statin dose</i>	1.04; 0.92-1.17	1.02; 0.85-1.23	1.01; 0.79-1.28
<i>Statin duration</i>			
<30 days	1.04; 0.43-2.51	0.31; 0.04-2.21	0.29; 0.04-2.04
31-365	2.02; 1.48-2.74	1.66; 1.11-2.49	1.70; 1.13-2.56
>365 days	1.51; 1.21-1.89	1.51; 1.15-1.98	1.37; 1.04-1.81
Never statin users	Ref		

Abbreviation: HR, hazard ratio; CI, confidence interval

Model 1 adjusted for age, gender, cohort (I, II and III), smoking status, alcohol consumption, physical activity, and education level, and model 2 included all variables in model I addition to body mass index and hypertension.

*Others including pravastatin and fluvastatin.

Table 4. Sensitivity analyses on the associations of statin ever use with incident type 2 diabetes

	Model 2: HR; 95% CI
Excluding cases with impaired fasting glucose at baseline	1.28; 1.00-1.76
Model 2+TC	1.42; 1.12-1.80
Model 2+HDL-C, LDL-C and TG	1.26; 0.99-1.61
Model 2+F.H.D	1.26; 0.98-1.62
Model 2+ PPIs	1.30; 1.03-1.65
Model 2+ fasting serum glucose and insulin concentrations	1.41; 1.12-1.78
Interaction between statin ever use and serum fasting glucose concentrations	3.51; 2.51-4.91

Abbreviations: HR, hazard ratio; CI, confidence interval; TC, total cholesterol; HDL-C, high density lipoproteins-cholesterol; TG, triglycerides; F.H.D, family history of diabetes; PPIs, proton pump inhibitors.

Model 2 adjusted for age, gender, cohort (I, II and III), smoking status, alcohol consumption, physical activity, education level, body mass index and hypertension.

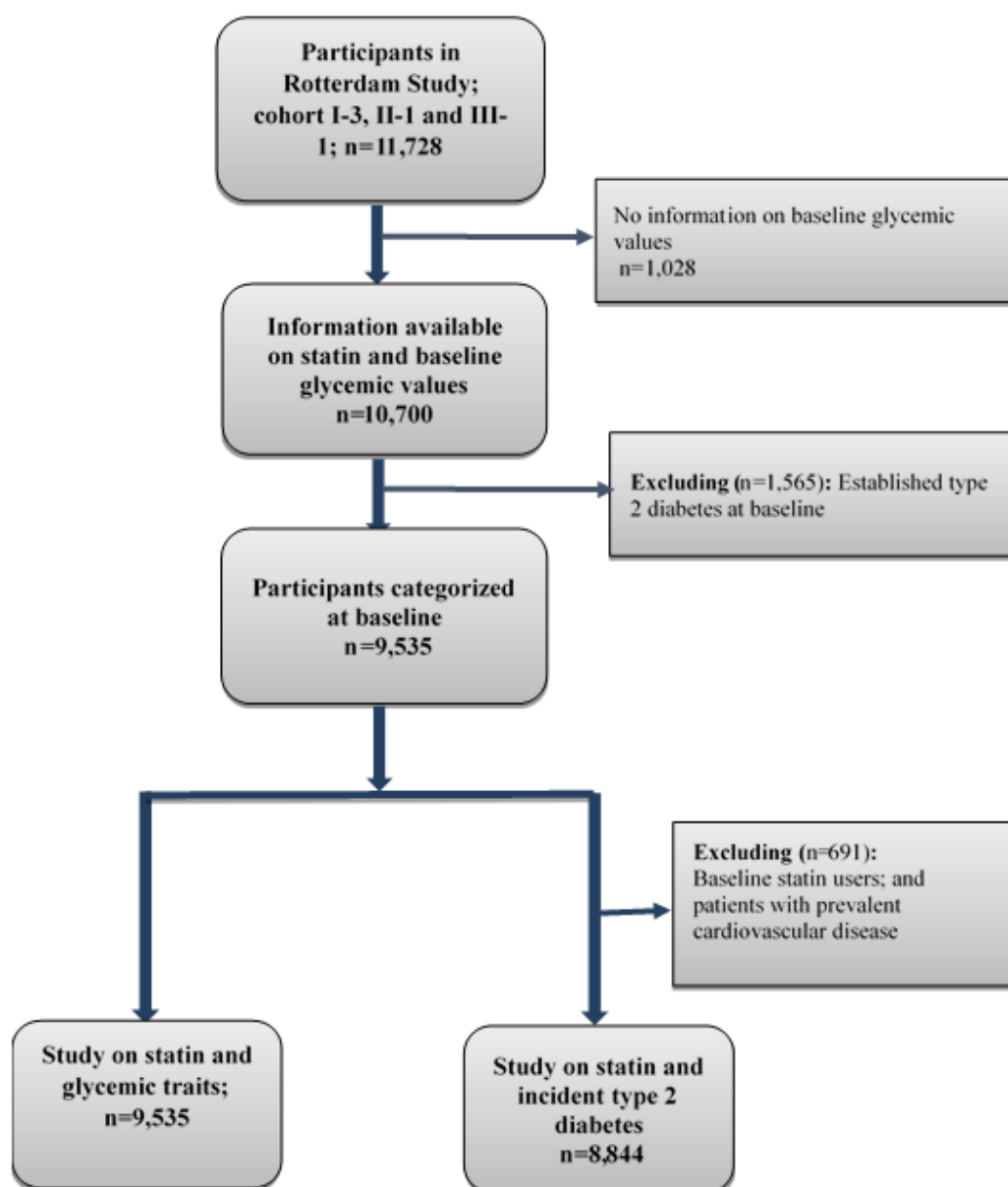


Fig 1. Flowchart of study population for the current study